UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Jan Vijg

Serial No. 09/306,333 Art Unit: 1643

Filed: May 6, 1999 Examiner: Souaya, Jehanne E.

For: BRCA 1 and bMLHI Gene Primer Sequences and Method for Testing

DECLARATION OF R. DAVID RINES

1. I am R. David Rines (Bowdoin College), the earlier affiant of a declaration filed herein on or about October, 2000. I have served as a researcher with the applicant, Dr. Jan Vijg, in his inventions and methods of TDGS electrophoresis described in the references cited as prior art by the Office in the present application ("Vijg" and "Vijg II"), from 1995 through 1998 at the Beth Israel (–Deaconess) Medical Center-Harvard Medical School, and then at Accelerated Genomics, Inc., continuing the same work, at San Antonio.

2. On page 13 of its outstanding Office action herein, the Examiner correctly notes that "Vijg teaches such a method with regard to the Rb gene"; "Vijg II teaches an example with p53"; and a method that may be tried with other genes, as well.

3. Up until the time we decided to tackle the BRCA1 gene, however, all of our experience and all of the teachings in "Vijg" and Vijg II" dealt with gene exons of the order of up to several hundred b, and we had developed the methodology of sub-division, where required, into overlapping fragments to cover the melting profile of such order of magnitude of exons in RB, p53, and MLH1, also in the above application. For the MLH1 of the present application, for example, the specification of the above application described

> "exons 11 and 12 had to be subdivided into overlapping fragments, two multiplex maps...being used, with the long PCR carried out as a four-plex PCR". (page 3).

4. We had never before encountered a gene having a single exon that was an order of magnitude larger than (more than *ten times*) the largest exons of our experience and that of the Vijg references, and certainly not one where a single exon contained more than a majority of the whole coding region; i.e., with the BRCA1, as stated in the original specification of the above application (page 3), a totally unique

> "exon 11 (which) contains approximately *60%* of the coding region".

5. While, together with Dr. Vijg, as "experts" in this Vijg methodology, not merely workers of "ordinary skill", and though aided by the use of the computer methodology of "Vijg II" to get a rough starting point, we had great difficulty in finally reaching appropriate primer and clamping sequences for covering the unprecedented wide melting domains of this giant exon 11, so as to be substantially 100% sure we would not overlook a single mutation.

6. We found that our expectation that the type of subdivision philosophy we had earlier consistently used ("Vijg" and "Vijg II") for overlapping fragments in one or two multiplex groups, (two multiplex groups for the MLH1 of this same patent application), just would not

work for the BRCA1. It required an uncontemplated manipulation, revision and unexpected departure from this prior sub- division philosophy and teaching to solve the problem. In the words of the *original* specification (page 3)

> "Using the long PCR amplicons as template, all 24 exons were amplified in a total of 37 fragments distributed over *5 multiplex groups*".

And, this ultimate radical departure from the simple overlap of our prior experiences with the Vijg technique is also described in the *original* specification:

> "The overlap and sometimes short distances from fragment to fragment *necessitated* the use *of so many multiplex short PCR groups*".

7. Another departure from the published procedures and basic computer model of "Vijg" and "Vijg II" that was ultimately found to be required for attaining satisfactory primer and clamp sequences for BRCA1 mutation detection, was that

> "The non-coding exons 1a, 1b and the non-coding part of exon 24 *were excluded.* (page 3).

We did not know this *a priori* and it certainly was not taught or inherent in "Vijg" and "Vijg II".

8. Still another departure from the teachings of "Vijg "and "Vijg II" that evolved during our almost one year's experimentation to achieve the working invention herein claimed, and with the repeatable reliability and improved resolution and clarity mentioned in the earlier Rines Declaration, was the reversal of the order of emphasis in primer trials to accommodate *first* the various melting profile considerations of the huge 3400b exon 11, and *then* to proceed with PCR conditions, rather than in the opposite order as taught in "Vijg" and "Vijg II".

9. Though using "Vijg" and "Vijg II" as our starting points, together with Dr. Vijg himself, the required modifications of the original "Vijg" and "Vijg II" procedures above-discussed for

ultimately achieving the specific successful sequences claimed in the above application, were far from "obvious" to us. They required long and sometimes counter-intuitive experimentation which we submit would hardly have been evident to an ordinary skilled artisan.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Sec. 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: December 18, 2003

R. David Rines